

**The Relation between Vitamin D Level
and Pregnancy Outcome in Women with
Unexplained Infertility Undergoing
Induction of Ovulation**

Thesis

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in Obstetrics and Gynecology*

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Contents

<i>Subject</i>	<i>Page</i>
List of Abbreviations	I
List of Tables	III
List of Figures	V
Protocol	-
Introduction	1
Aim of the Work	4
Chapter (1): Unexplained Infertility.....	5
Chapter (2): Vitamin D	21
Chapter (3): Vitamin D and Reproduction.....	36
Patients and Methods	43
Results	54
Discussion	74
Summary	84
Conclusion	89
Recommendations	90
References	91
Arabic Summary	-

List of Abbreviations

Abb.	Full term
ACOG	American College of Obstetrics and Gynecology
AIs	Aromatase inhibitors
AMH	Anti-müllerian hormone
ART	Assisted reproductive technologies
ASRM	American Society of Reproductive Medicine
AUC	Area under the ROC curve
BMI	Body mass index
CC	Clomiphene citrate
COH	Controlled ovarian hyper-stimulation
DBP	Vit. d binding protein
ELISA	Enzyme-linked immunoassay
FAI	Free androgen index
FDA	Food and Drug Administration
FHA	Functional hypothalamic amenorrhea
FSH	Follicle-stimulating hormone
GDM	Gestational diabetes mellitus
GnRH	Gonadotropin-releasing hormone
HA	Hypothalamic amenorrhea
hCG	Human chorionic gonadotropin
hMG	Human menopausal gonadotropins
ICI	Intracervical insemination
IOM	Institute of Medicine
IUI	Intrauterine insemination
IV	Intravenous

 List of Abbreviations

Abb.	Full term
IVF	In vitro fertilization
LH	Luteinizing hormone
MED	Minimal erythematous dose
MRI	Magnetic resonance imaging
NIH	National Institutes of Health
OHSS	Ovarian hyperstimulation syndrome
OI	Ovulation induction
PASS	Power Analysis and Sample Size Software
PCOS	Polycystic ovary syndrome
POI	Primary ovarian insufficiency
PPCOS II	Pregnancy in Polycystic Ovary Syndrome II
PTH	Parathormone
PTHrP	Parathyroid hormone-related protein
rhFSH	Recombinant human follicle-stimulating hormone
ROC	Receiver-operating characteristic
SA-HRP	Streptavidin-horseradish peroxidase
SE	Standard error
SERMs	Selective estrogen receptor modulators
SHBG	Sex hormone-binding globulin
TMB	Transient monocular blindness
uFSH	Urinary follicle-stimulating hormone
UVB	Ultraviolet b
VDD	Vitamin D deficiency
VDR	Vitamin D receptor
Vit	Vitamin
WHO	World Health Organization

List of Tables

No	Table	Page
1	Natural sources vitamin D content	22
2	Dietary reference intakes and maximum tolerable intakes of vitamin D in different stages of life - IOM, 2010	32
3	Demographic characteristics whole study population	55
4	Hormonal profile for study women	55
5	Ultrasound workup for study women and duration of induction	56
6	Vitamin D level and status among study women	57
7	Pregnancy rate among study women	60
8	Comparison of women with positive or negative pregnancy test	61
9	Vitamin D status in women with positive or negative pregnancy test	63
10	Type of infertility in women with positive or negative pregnancy test	66
11	Receiver-operating characteristic (ROC) curve analysis for prediction of positive chemical pregnancy	67
12	Correlation between Vitamin D level and other numerical variables	69
13A	Multivariable binary logistic regression	71



List of Tables

No	Table	Page
	analysis for the relation between Vitamin D level and biochemical pregnancy as adjusted for age and type and duration of infertility	
13B	Multivariable binary logistic regression analysis for the relation between Vitamin D status and biochemical pregnancy as adjusted for age and type and duration of infertility	72

List of Figures

No	Figure	Page
1	Physiology and vitamin D metabolism	25
2	Schematic representation of the major causes of vitamin D deficiency and potential health consequences	29
3	Measure of the range of variation of red blood cell volume Functions of 1.25-dihydroxy vitamin D	31
4	Box plot illustrating distributon of vitamin D level in the whole study cohort	58
5	Vitamin D status in the whole study cohort	59
6	Prevalence of Vitamin D deficiency (<20 ng/ml) in the whole study cohort	59
7	Chemical pregnancy rate in the whole study cohort	60
8	Mean vitamin D level in patients with positive or negative chemical pregnancy	62
9	Vitamin D status in patients with positive or negative chemical pregnancy	64
10	Prevalence of Vitamin D deficiency (<20 ng/ml) in patients with positive or negative chemical pregnancy	65
11	Type of infertility in patients with positive or negative chemical pregnancy	66
12	Receiver-operating characteristic (ROC) curve analysis for prediction of positive chemical pregnancy using serum Vitamin D level	68
13	Scatter plot illustrating the correlation between serum vitamin D and LH level	70

PROTOCOL OF A THESIS FOR PARTIAL FULFILMENT OF MASTER DEGREE IN OBSTETRICS & GYNAECOLOGY

Title of the Protocol: The Relation between Vitamin D Level and Pregnancy Outcome in Women with Unexplained Infertility Undergoing Induction of Ovulation

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What is already known on this subject? AND What does this study add?

Vitamin D deficiency is directly responsible for a reduced fertility and reproduction capacity. Women with higher level of Vitamin D in serum and follicular fluid are more likely to become pregnant. This cohort study will assess the effect of serum level of Vitamin D on pregnancy outcome in women with unexplained infertility undergoing an induction of ovulation.

1.INTRODUCTION/ REVIEW

Infertility is a unique medical condition because it involves a couple, rather than a single individual. It is defined as inability of a couple to conceive after 12 months of regular intercourse without use of contraception in women less than 35 years of age; and after six months of regular intercourse without use of contraception in women 35 years and older (*ASRM, 2008*).

Unexplained infertility refers to the absence of a definable cause for a couple's failure to achieve pregnancy after 12 months of attempting conception despite a thorough evaluation, or after six months in women 35 and older (*ASRM, 2008*).

Several possibilities have been proposed to explain why some couples fail to conceive in the absence of an identifiable cause. Subtle changes in follicle development, ovulation, and the luteal phase have been reported in some of these women (*Blacker et al., 1997*).

Vitamin D has profound effects on many biological systems, including the reproductive system (*Rudick et al., 2011*).

The spectrum of Vitamin D target organs has expanded and the reproductive role of Vitamin D is highlighted by expression of the

Vitamin D receptor (VDR) and enzymes that metabolise Vitamin D in testis, the male reproductive tract and human spermatozoa (**Jensen, 2014**).

The presence of Vitamin D receptor (VDR) in glandular epithelial cells of endometrium, granulosa cells, fallopian epithelial cells and cumulus oophorus cells of ovary has been confirmed (**Aleyasin et al., 2011**).

Different studies have indicated that Vitamin D deficiency is directly responsible for a reduced fertility and reproduction capacity in female rat (**Anifandis et al., 2010; Rudick et al., 2012**).

Some studies on the Vitamin D status of women undergoing controlled ovarian hyper-stimulation (COH) for Assisted Reproduction Technologies have been done. Results showed that Vitamin D deficiency is highly prevalent among women undergoing COH, ranging from 21% to 31% across studies conducted in Western countries and reaching 75–99% in Iranian studies and showed that Vitamin D supplementation increase fertility and reproduction capacity (**Vanni et al., 2014**).

Women with higher level of Vitamin D in serum and follicular fluid are more likely to develop a pregnancy following in vitro fertilisation (**Ozkan et al., 2010**).

Literature review shows that Vitamin D is involved in many functions of the human reproductive system in both genders, but no complete analysis of the potential relationship between Vitamin D status and pregnancy rate parameters is currently available.

2.AIM / OBJECTIVES

This cohort study will assess the association between serum level of Vitamin D and pregnancy outcome in women with unexplained infertility undergoing an induction of ovulation. i.e. prediction of response to clomiphene citrate induction of ovulation for women with unexplained infertility.

Study hypothesis: In women with unexplained infertility undergoing an induction of ovulation, there is association between serum level of Vitamin D and pregnancy outcome.

Null hypothesis: In women with unexplained infertility undergoing an induction of ovulation, there is no association between serum level of Vitamin D and pregnancy outcome.

Study question: In women with unexplained infertility undergoing an induction of ovulation, is there association between serum level of Vitamin D and pregnancy outcome?

3.METHODOLOGY:

Type of Study: Cohort study.

Study Setting: The study will be conducted at Ain Shams University Maternity Hospital.

Study Population: The study will be conducted at Ain Shams University Maternity Hospital (infertility clinic).

Inclusion criteria:

Women with unexplained infertility with the following criteria:

1. Women in childbearing period
2. Husband normal semen analysis: The World Health Organization (WHO) has published revised lower reference limits for semen analyses. The following parameters represent the generally accepted 5th percentile: (Cooper *et al.*, 2010)

- Volume: 1.5 mL
 - Sperm concentration: 15 million spermatozoa/mL
 - Total sperm number: 39 million spermatozoa/ ejaculate
 - Morphology : 4 % normal forms
 - Vitality: 58 % live
 - Progressive motility : 32 %
 - Total motility (progressive and nonprogressive): 40 %
3. Regular menstrual cycle.
 4. Normal transvaginal ultrasonography.
 5. Normal HSG and/or laparoscopy with chromotubation.
 6. Normal hormonal profile for FSH, LH, Prolactin and thyroid gland.

Exclusion criteria:

1. Women with diminished ovarian reserve as denoted by a serum FSH > 10 mIU/ml or a serum AMH < 1 ng/ml.
2. Women with body mass index 35 kg/m² or more.
3. Women who have clomiphene citrate (CC) resistance, or who had prior history of serious adverse effects with clomiphene citrate (CC) (blurring of vision, or severe OHSS).
4. Women with expected decreased endometrial receptivity (e.g. intra uterine abnormalities: adhesions or chronic endometritis).
5. Women with endocrinal disorders as hyperprolactinemia and thyroid dysfunction or chronic illness as active liver diseases and renal disease.
6. Women with documented pelvic diseases as endometriosis, any ovarian pathology, hydro- or pyo-salpinx and uterine fibroids, previous history of ovarian drilling.
7. Women consuming drugs interfering with Vitamin D metabolism
8. Women who refused to participate.

Study Procedures: This will be cohort study of women with unexplained infertility who will undergo induction of ovulation at Ain Shams University Maternity Hospital (infertility clinic).

Study intervention

For All selected women, the following will be done:

- Detailed history.
- Physical examination including general, abdominal and local examination.
- Baseline pelvic Ultrasound.
- Baseline serum FSH, LH, E2, free testosterone, estradiol, TSH, free T3 and T4 and prolactin, HSG, Semen analysis to rule out male factor. Vitamin D status will be measured by assessing circulatory levels of 25(OH)D using an enzyme immunoassay. Levels of 25(OH)D will be determined by using 25-OH Vitamin D ELISA kit (Euroimmun, Luebeck, Germany) according to manufacturer's instructions. The serum 25(OH)D will be categorized according to clinically accepted ranges for Vitamin D deficiency (<20 ng/ml), insufficiency (20–30 ng/ml) and replete (>30 ng/ml) (*Zarnani et al., 2010*).
- Clomiphene Citrate 50mg tablet will be taken orally for 5 days, from cycle day 3 to cycle day 7.
- Transvaginal ultrasound folliculometry will be done at ultrasound unit by expert doctor using (**Samsung H 60**) at 9th day of menstrual cycle, then follow up every other day until the mean diameter of the largest follicle reaches 18mm, Triggering ovulation will be made by intramuscular injection of 10,000IU human chorionic gonadotropin (HCG; Profasi HP®, Serono S. A., Geneva, Switzerland) when the leading follicle reached ≥ 18 mm diameter.
- Free sexual intercourse will be encouraged from the day of HCG administration only in cases when no more than three

follicles bigger than 17mm are observed; then, HCG injection will not be administered and protected sexual intercourse will be recommended in order to avoid high order multiple conception.

- The number and size of follicles will be calculated, the follicle will be identified and displayed in its maximum diameter, two dimensional measurements will be made, and their mean will be taken as the true follicle diameter, then the endometrial thickness in mm will be measured in the sagittal view as the maximum thickness between the highly reflective interface of the endometrial-myometrial junction and the mean endometrial thickness will be taken in day of HCG injection.
- When 2-3 follicles with 18-30 mm diameter will be visible, an injection of HCG will be performed.
- If no dominant follicle detected till day 15 of the cycle, transvaginal ultrasound will be continued every other day till day 20 and the endometrial thickness will be measured, if still no dominant follicle till that time, patient will be considered as failed induction.
- Diagnosis of pregnancy will be done by performing qualitative serum β HCG one week after missed period.

Sample Size: This study will be conducted on 143 women.

Sample Justification: The required sample size has been calculated using the PASS 15 Power Analysis and Sample Size Software (PASS©) version 15 (2017); NCSS, LLC. Kaysville, Utah, USA.

The primary outcome measure is the clinical pregnancy rate. There is at present limited information regarding the relation between vitamin D levels and occurrence of clinical pregnancy in patients undergoing induction of ovulation for unexplained infertility. So, the present study will target an effect size that is clinically relevant.

A previous study reported that approximately 46% of patients undergoing induction of ovulation for unexplained infertility had